UTERINE BIOLOGY OF THE PIG: GENE EXPRESSION FOR ESTABLISHMENT OF PREGNANCY

An Undergraduate Research Scholars Thesis

by

GLADYS BROWN

Submitted to Honors and Undergraduate Research
Texas A&M University
In partial fulfilment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor Dr. Fuller W. Bazer

May 2014

Major: Biomedical sciences

TABLE OF CONTENTS

ABSTRACT

Uterine Biology of the Pig: Gene Expression for Establishment of Pregnancy. (May 2014)

Gladys Brown
Department of Biomedical Sciences
Texas A&M University

Research Advisor: Dr. Fuller W. Bazer
Department of Animal Science

Pigs have considerable economic benefits so the death of 20 to 40 percent of their embryos during pregnancy represents a major economic loss [1]. Therefore, identifying the expression of key genes associated with embryonic development and their roles during pregnancy is essential. Estrogen produced by the embryonic membranes of the conceptus (embryo and its extra-embryonic membranes) between Days 11 through 13 and then Days 15 through 25 of gestation in the pig act with prolactin from the anterior pituitary gland of the maternal system for pregnancy recognition signaling to maintain functional corpora lutea on the ovaries to produce progesterone, the hormone of pregnancy. The effect of the endogenous estrogens can be mimicked by injecting exogenous estrogen on Days 11 through 15 of the estrous cycle. Estrogen increases expression of receptors for prolactin. Understanding the interactions between conceptus and uterine endometrium is required for successful establishment of pregnancy and induction of expression of genes is critical for conceptus survival and development.

ACKNOWLEDGEMENT

My special thanks go to God for giving me the opportunity to participate in this program. I also show my gratitude to my research advisor Dr. Bazer for giving me the privilege to work in his laboratory, his help and support throughout this journey. Other thanks go to family, friends and love ones.

CHAPTER I

INTRODUCTION

Estradiol is the pregnancy recognition signal in pigs [2, 3]. However, the pig conceptus also secretes both interferon gamma (IFNG) and interferon delta (IFND) during the period of pregnancy recognition, but they are not the pregnancy recognition signal. Although interferons have biological effects critical to immune responses that protect the body against viral infections, the roles of IFNG and IFND in the pregnant uterus of pigs are not known. Estrogen produced by pig conceptuses between Days 11 to 13 and then Days 15 through 25 of gestation act with prolactin from the maternal anterior pituitary gland for pregnancy recognition signaling. Estrogen has the effect of increasing expression of receptors for prolactin.

Oxytocin, a hormone released by the posterior pituitary gland (pars nervosa), induces contractions of the uterus during labor, but also has important implications due to its presence in uterine secretions of pigs and the uterine endometrium is responsive to oxytocin in pigs [4-6]. The development of endometrial responsiveness to oxytocin occurs between Days 12 and 14 of the estrous cycle and administering oxytocin during late diestrus can cause premature regression of the ovarian corpora lutea (CL). It is of interest that the uterine endometrium of pigs, which is the primary source of oxytocin during late diestrus, secretes more oxytocin than the pars nervosa during diestrus [4-6]. Furthermore, it is also known that there is greater secretion of oxytocin in the early stages of pregnancy in pigs and this increase in secretion of oxytocin coincides with the period of rapid elongation of the pig conceptus and its production of estrogen for pregnancy recognition signaling [7-9]. Progesterone from the ovarian corpora lutea is the hormone of

pregnancy that acts through progesterone receptors (PGR) in the uterine epithelia and other target cells; however, PGR must be down-regulated in uterine epithelia in order for pig conceptuses to undergo implantation between Days 13 and 25 of pregnancy. Pig blastocysts undergo a dramatic transition from 10-15 mm diameter between Days 10 and 12 of pregnancy to achieve a length of 800 to 1000 mm between Days 12 and 15 of pregnancy. This rapid development establishes an extensive surface area of contact between trophectoderm and the uterine luminal epithelium for transport of nutrients and oxygen to the conceptus. The trophectoderm secretes estrogen between Days 11 and 25 of early pregnancy, while IFNG and IFND are secreted primarily between Days 13 and 18 of pregnancy. Estrogen modulates the expression of genes responsible for endometrial remodeling for implantation between Days 13 and 25 of gestation and the messenger RNAs for IFNG and IFND are abundant in the trophectoderm between Days 13 and 18 of pregnancy. By understanding the effects of oxytocin on pregnant and cyclic uteri, we can hypothesized that there will be greater expression of oxytocin and oxytocin receptors in uteri of pregnant as compared to cyclic (nonpregnant) pigs.

CHAPTER II

MATERIALS AND METHODS

Animals and tissue collection

Uteri obtained from sexually mature female pigs (gilts) were obtained on Days 11, 13, 15 and 17 of pregnancy and the estrous cycle was used for this experiment. The samples were obtained from the United States Department of Agriculture Meat Animal Research Center, Clay Center Nebraska. All experimental and surgical procedures were in compliance with the Guide for Care and Use of Agricultural Animals in Teaching and Research.

Experimental design

Study: To evaluate the effects of day and pregnancy status on expression of oxytocin and oxytocin receptors in uteri of pregnant and cyclic gilts. The gilts were oophorectomize-hysterectomized on Day 11, 13, 15 or 17 of the estrous cycle and Day 11, 13, 15 or 17 of pregnancy.

Immunohistochemical analyses

After the gilts were slaughtered, , the uterine tissue was collected and placed in a tube containing fresh 4% paraformaldehyde and placed on a rocker overnight for fixation of the tissue. Once the period of fixation was finished, the tissue was rinsed in phosphate buffered saline (PBS), and then processed through a series of ethanol washes to displace the water. The tissue was then infiltrated with and embedded in paraffin. The samples of uterine tissue from each gilt were cut into 5 micron sections that were placed on a microscope slide. During this process, the tissue on

the slides was first deparaffinize and then rehydrate through ethanol and then to the buffer solution. Antigen retrieval was performed using the boiling citrate method. The slides were placed in a boiling citrate buffer solution and incubate for 10 minutes and allowed to cool for 20 minutes. Endogenous peroxidase activity was blocked by mixing 1% hydrogen peroxide in methanol (6.7ml 30% hydrogen peroxide per 200ml methanol) and tissues were then allowed to sit for 15 minute at room temperature before being rinsed twice in a buffer solution. The slide were then blocked by removing excess liquid and adding diluted protein blocker from the Vectastain ABC Kit including rabbit IgG to each section.. The ABC reagent is used to detect any molecule that is biotinylated. Reagent A (Avidin DH was modified using a proprietary process to remove non-specific binding). Reagent B (biotinylated peroxidase with an enhanced enzyme for biotin) was prepared by adding 3 drops protein blocker concentrate to 10ml of PBS. The next step was to proceed with the primary antibody. The slides were incubated overnight in a humidified chamber and then washed twice with PBS. Secondary antibody was prepared by adding 3 drops of protein blocker and 1 drop of biotinylated secondary antibody stock to 10 ml buffer solution and incubating the tissue for 60 minute at 37 degree Celsius in a humidified chamber. The slides are then rinsed twice in a buffer solution for 5 minutes each. A reagent detector was then prepared by adding 2 drops of reagent A followed by reagent B and tissue allowed to stand for 30 minutes at room temperature. After that, the diluted detector reagent was added to the slides, incubated for 30 minute at 37 Celsius and rinsed twice afterwards in a buffer solution for 5 minutes each. The slides were then washed with 0.05M Tris –hydrochloric acid (Tris-HCl) for 5 minute. A 3, 3-diaminobenzidine hydrochloride hydrate (DAB) solution is prepared by adding hydrogen peroxide to 100mg of DAB and 200 ml of 0.05 M Tris-HCl solution immediately before adding the solution to the slides. The slides were then incubated in

the DAB for 2-4 minutes in order to visualize antibody binding. The slides were evaluated microscopically to determine staining strength. After that, the slides were rinsed in tap water to stop the reaction. Slides were then dehydrated through alcohol to Citrisolve. Coverslip were then affixed with permount and placed in a box due to the sensitivity of DAB.

CHAPTER III

RESULTS

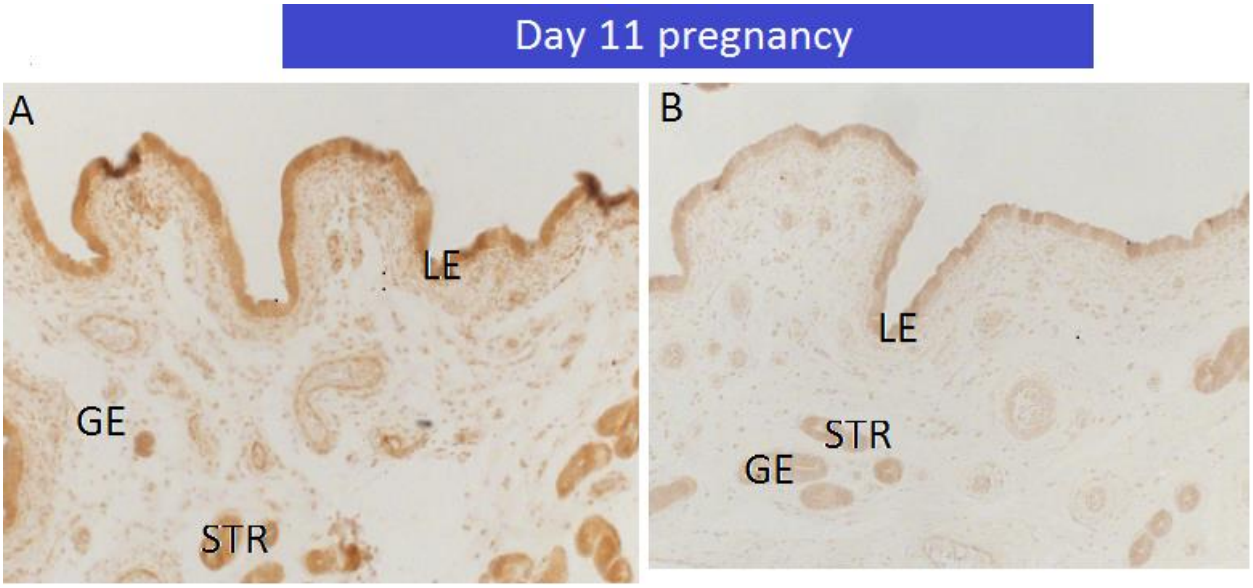

Fig. 1.A. Expression of oxytocin receptor (OXTR) protein was detected in uterine lumenal (LE) and glandular (GE) epithelial in response to the antibody to OXTR. B. OXTR protein was not detected by an irrelevant control IgG. The stromal (STR). Width, 900 μm.

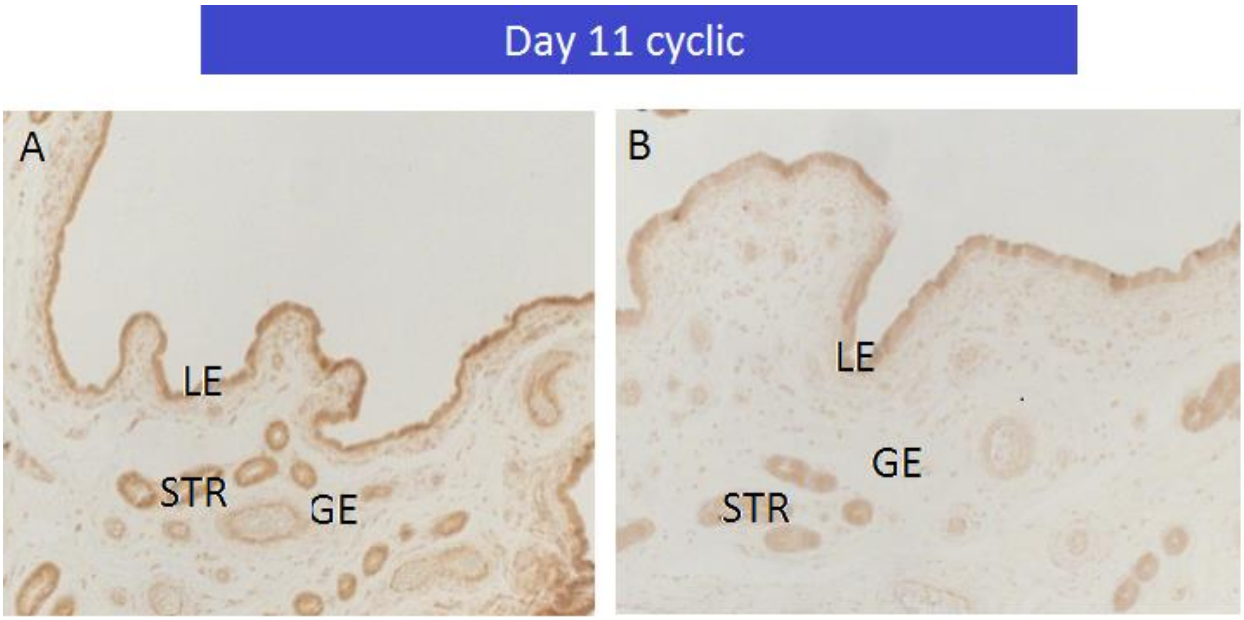

Fig. 2.A. Expression of OXTR was detected in uterine LE and GE of the cyclic gilts, but strength of staining was less than for Day 11 pregnant gilts as shown in Fig. 1A. B. OXTR protein was not detected by irrelevant control IgG. Width, 900 μm.

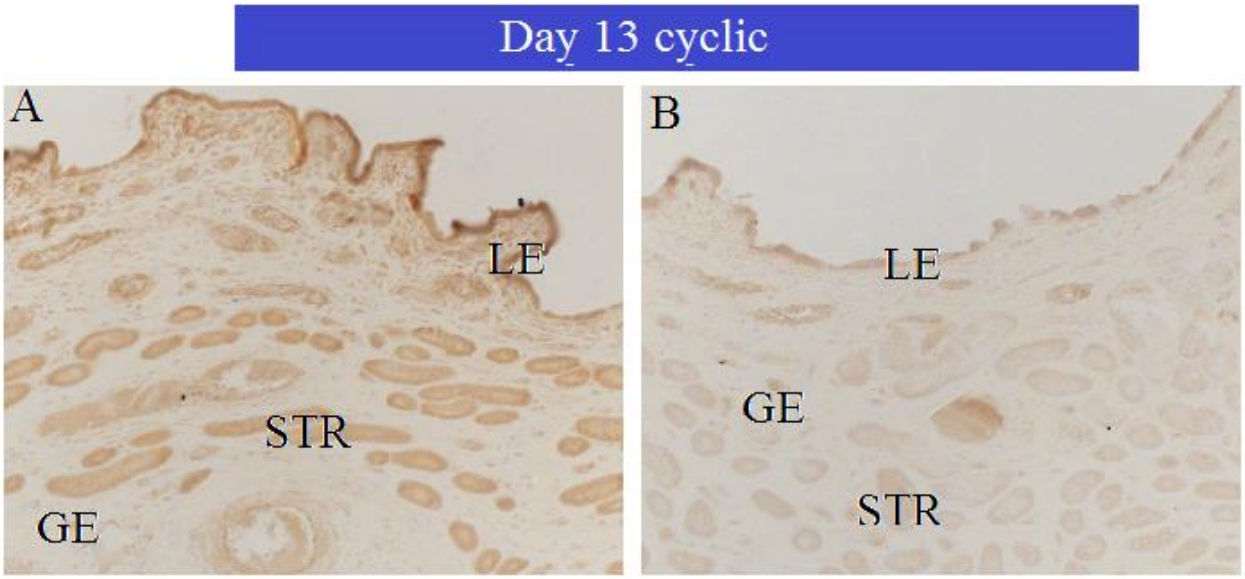

Fig. 3. A. Expression of OXTR was more abundant in uterine luminal (LE) than glandular (GE) epithelial cells. B. OXTR protein was not detected by an irrelevant control IgG. Width, 900 μm.

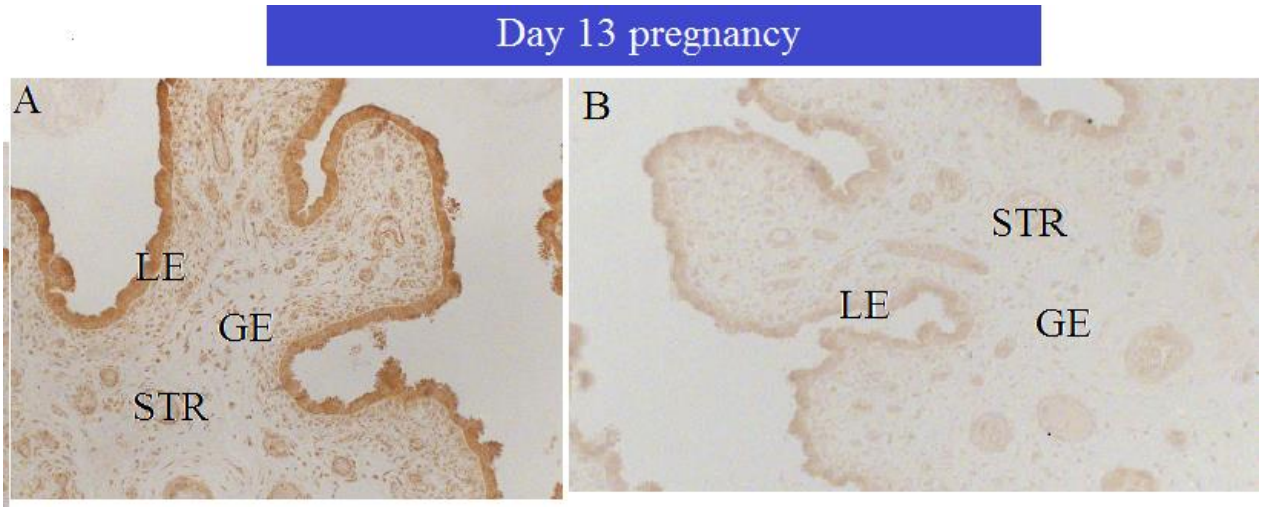

Fig.4. A. Expression of OXTR was strong in uterine LE as compared to GE on Day 13 of pregnancy. B. OXTR protein was not detected by irrelevant control IgG. Width, 900 μm.

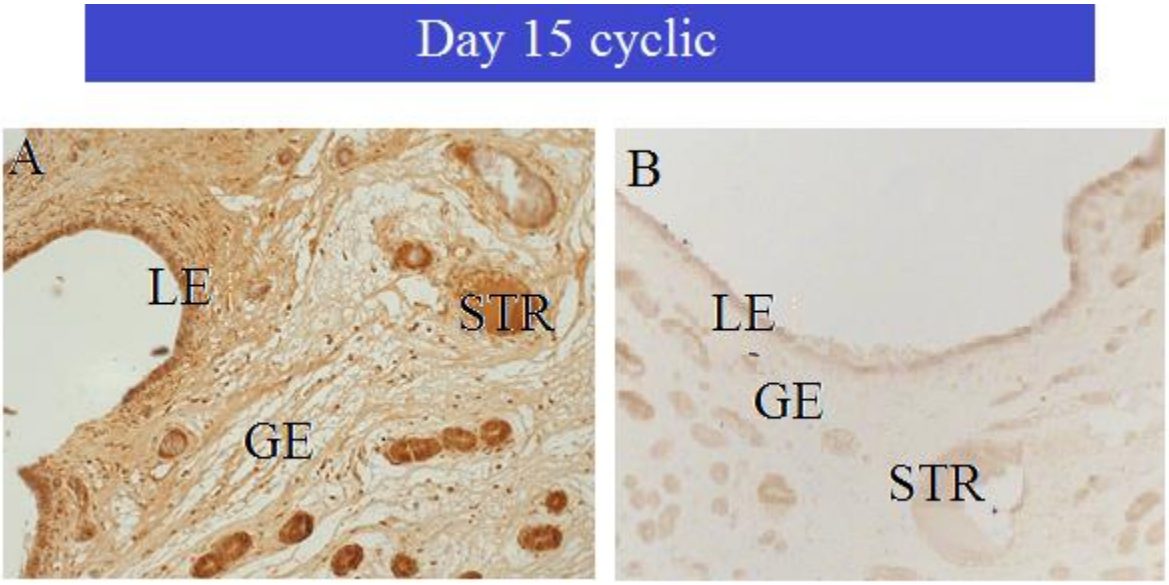

Fig. 5. A. OXTR expression is strong in both uterine LE and GE, as well as in stromal cells beneath the LE. B. OXTR protein was not detected by irrelevant control IgG. Width, 900 μm.

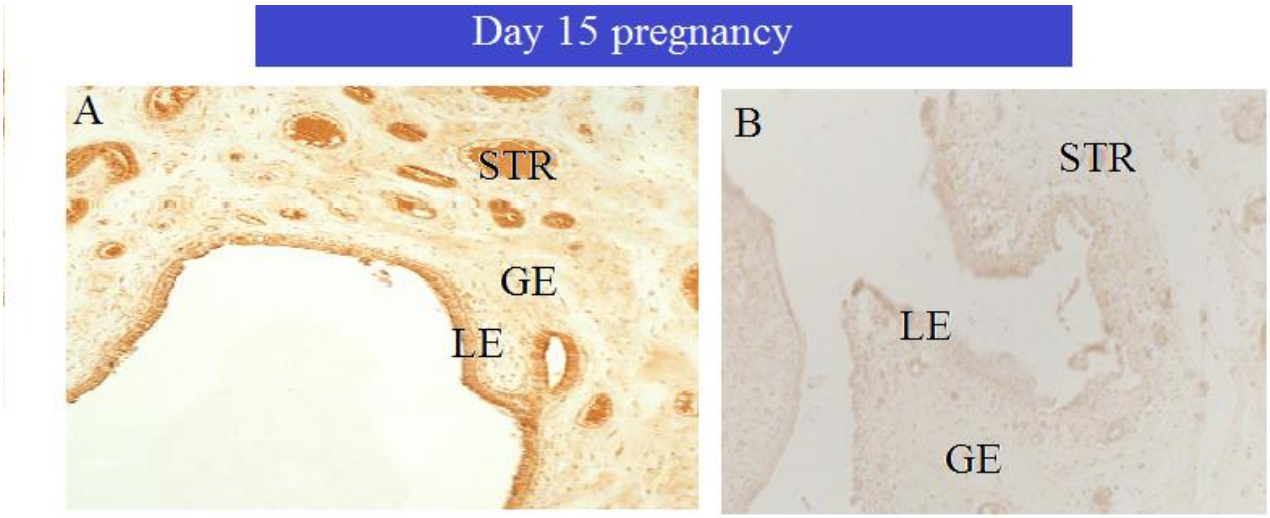

Fig. 6. A. There was strong staining for OXTR in uterine GE and LE. B. OXTR protein was not detected by irrelevant control IgG. Width, 900 μm.

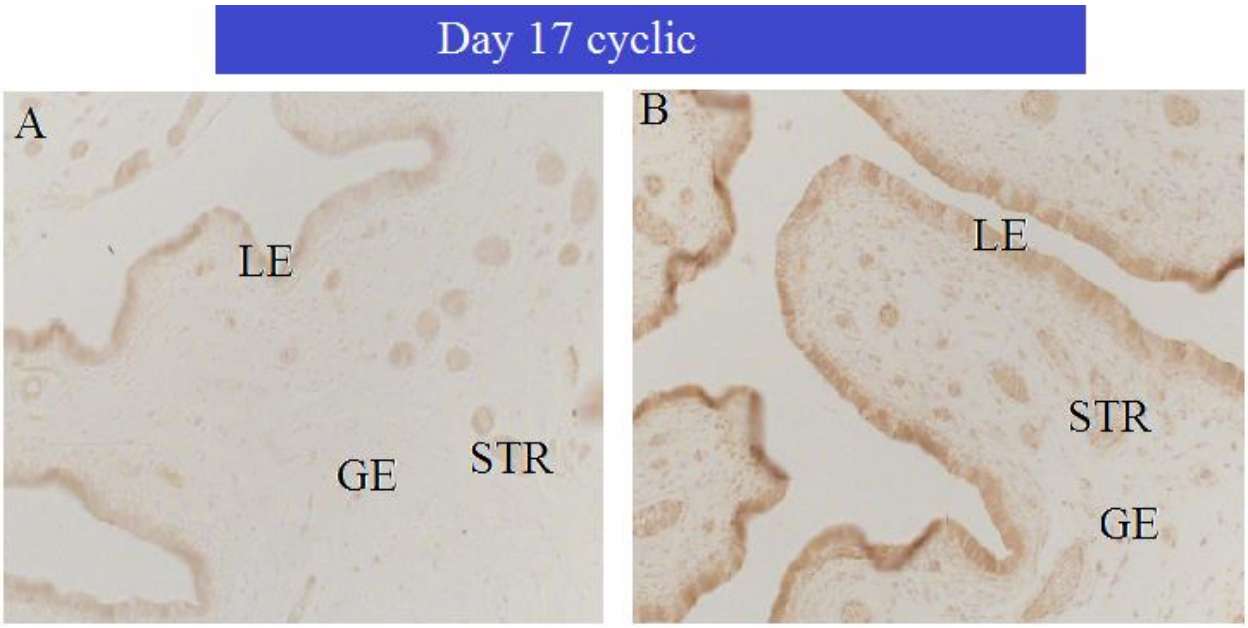

Fig.7. A. Expression of OXTR is detectable in uterine LE, but weak in uterine GE and much less than for Days 13 and 15 of the estrous cycle. b. OXTR protein was not detected by irrelevant control IgG. Width, 900 μm.

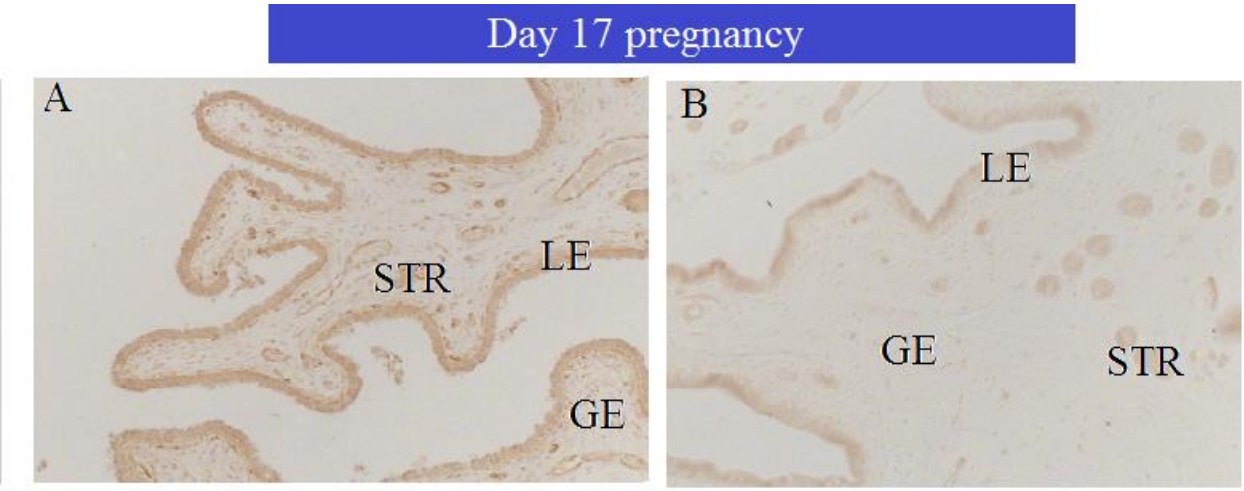

Fig 8. A. expression of OXTR in uterine LE and GE is much less abundant than on Days 13 and 15 of the estrous cycle and pregnancy. B. OXTR protein was not detected by irrelevant control IgG. Width, 900 μm.

CHAPTER IV

CONCLUSION

These results indicate that oxytocin receptor expression is detectable in uterine luminal and glandular epithelia of cyclic and pregnant gilts on Days 13, 15 and 17 after onset of estrus; however, the relative abundance decreases in both cyclic and pregnant gilts between Days 15 and 17 after onset of estrus. The weak expression of oxytocin receptor protein on Day 17 of the estrous cycle and pregnancy may reflect the loss of expression of receptors for estradiol or the significant increase in expression of receptors for progesterone, either of which may preclude expression of the oxytocin receptor gene.

REFERENCES

1. Bazer FW. Pregnancy recognition signaling mechanisms in ruminants and pigs. J Anim Sci Biotech 2013; 4:23.

2. Bazer FW. Mediators of maternal recognition of pregnancy in mammals. Proc Soc Exp Biol. Med 1992; 199:373-384.

3. Sample GL, Blackwell DM, Kubotsu SL, Mirando MA. Endocrine secretion of prostaglandin F2alpha in cyclic gilts is decreased by intrauterine administration of exogenous oxytocin. Anim Reprod Sci 2004; 84:395-406.

5. Kotwica G, Dusza L, Ciereszko R, Okrasa S, Schams D.1990. Oxytocin plasma levels during spontaneous and cloprostenol-induced luteolysis in sows. Anim Reprod Sci 22:109–119.

6. Pitzel L, Welp K, Holtz W, König A. 1984. Neurohypophyseal hormones in the corpus luteum of the pig. Neuroendocrinol 6:1–5.

7. Trout WE, Smith GW, Gentry PC, Galvin JM, Keisler DH.1995. Oxytocin secretion by the endometrium of the pig during maternal recognition of pregnancy. Biol Reprod 52:189 (Abstract).

8. Vallet JL, Christenson RK, Trout WE, Klemcke HG.1998. Conceptus, progesterone, and breed effects on uterine protein secretion in swine. J Anim Sci 76:2657–2670.

9. Bazer FW, Thatcher WW. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2a by the uterine endometrium. Prostaglandins 1977; 14:397 401.